

# Desiccation survival

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 An abbreviated version of this protocol was published in eLIFE in Sep 2018

Humidity response depends on the small soluble protein Obp59a in *Drosophila*

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## Detailed protocol

1. Set up bottles of fly food to generate progeny
  - a. Use 10 virgin females and 5 males in each bottle as parents to control for crowding
  - b. Rear flies on standard cornmeal-dextrose agar food at 25 °C and 60% humidity
  - c. After 4 days, clear parents
2. For two days after eclosion, collect flies without using CO<sub>2</sub>
  - a. Transfer offspring to new bottles, adding ~200 flies per bottle to prevent overcrowding
  - b. Allow flies to mate for 24 hours
3. Make experimental arenas
  - a. Obtain 150 mm x 15 mm dishes with lids
  - b. Evenly space five 35 x 10 mm dishes within the larger dish and adhere to the bottom of the larger dish using double-sided tape
  - c. Remove and dispose of lids for the 35 x 10 mm dishes
  - d. Create arenas with defined relative humidity:
    - i. 0% RH produced by adding 25 mL Drierite around the smaller dishes
    - ii. 70% RH produced by adding 25 mL saturated NaCl around the smaller dishes
  - e. Equilibrate arenas in 25°C in the dark for ~10 hours
4. Separate males and females using CO<sub>2</sub>
  - a. Collect 20 sex-segregated flies in empty food vials
  - b. When all flies are separated, place all vials on ice to anesthetize flies
  - c. Swiftly knock flies into each small Petri dish
  - d. Use 20-25 replicates per condition (4-5 arenas)
  - e. Cover each small dish with mesh that has been autoclaved and cut into 50 x 50 mm squares
  - f. Seal the mesh around each small dish using zipties
  - g. Cover the arena using the lid, then seal with Parafilm
5. Count, blind to genotype and humidity conditions, the number of dead flies each hour for 12 hours

**How to cite:** (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Sun, J. S. (2019). Desiccation survival. Bio-protocol Preprint. [bio-protocol.org/prep22](https://bio-protocol.org/prep22).
2. Sun, J. S., Larter, N. K., Chahda, J. S., Rioux, D., Gumaste, A. and Carlson, J. R. (2018). Humidity response depends on the small soluble protein Obp59a in *Drosophila*. eLIFE. DOI: [10.7554/eLife.39249](https://doi.org/10.7554/eLife.39249)

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